


Remarks

This Amendment enters the references to SEQ ID NOs: 1-3 into the specification. The Amendment also cancels the second set of claims 54 and 55, and amends claims 1, 9, 13, 14, 16, 17, 21, 22, 24, 25, 27, 42, 43, 50, 54, 58 and 60. Support for the claim amendments can be found throughout the specification. No new matter has been added.

No fee is believed to be due in connection with this Amendment. However, if any fee is due, please charge our Deposit Account No. 06-1448.

Respectfully submitted,

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Replacement paragraphs showing the changes made therein:

Replacement paragraphs for page 4, lines 8-20:

One aspect of the present invention relates to isolated and/or recombinant forms of a cell- or tissue-specific F-box proteins, and portions thereof. For instance, there is provided isolated and/or recombinant polypeptides having an amino acid sequence identical or homologous (e.g., at least 65, 75, 85 or 95%) to the amino acid sequence as set forth in Figure 5B (SEQ ID NO: 2). The cell- or tissue-specific F-box polypeptide can have an amino acid sequence encoded by a nucleic acid which hybridizes under stringent conditions to the nucleotide sequence set forth in Figure 5A (SEQ ID NO: 1).

In another embodiment, other isolated and/or recombinant cell- or tissue-specific F-box polypeptides are provided, e.g., having an amino acid sequence identical or homologous (e.g., at least 65, 75, 85 or 95%) to the amino acid sequence set forth in Figure 5B (SEQ ID NO: 2). The cell- or tissue-specific F-box polypeptide can have an amino acid sequence encoded by a nucleic acid which hybridizes under stringent conditions to the nucleotide sequence set forth in Figure 5A (SEQ ID NO: 1).

Replacement paragraph for page 10, lines 25-28:

Figure 5 shows nucleotide (SEQ ID NO: 1) and amino acid (SEQ ID NO: 2) sequences of atrophin-1. A, shows the nucleotide sequence (SEQ ID NO: 1) of the mouse atrophin-1 gene. B, shows the deduced amino acid sequence (SEQ ID NO: 2) of mouse atrophin-1 protein. The F-box motif is underlined. C, shows a schematic representation of the atrophin-1 protein. The box represents the F-box motif.

Replacement paragraph for page 27, lines 16-32:

A "WD-40 motif", also referred to in the art as " β -transducin repeats" or "WD-40 repeats", is roughly defined as a contiguous sequence of about 25 to 50 amino acids with relatively-well conserved sets of amino acids at the two ends (amino- and carboxyl- terminal) of the sequence (reviewed in Simon et al., *Science* 252:802-808 (1991) and Neer et al., *Nature* 371:297 (1994)). Conserved sets of at least one WD-40 repeat of a WD-40 repeat-containing protein typically contain conserved amino acids at certain positions. The amino-terminal set, comprised of two contiguous amino acids, often contains a Gly followed by a His. The carboxyl-terminal set, comprised of six to eight contiguous amino acids, typically contains an Asp at its first position, and a Trp followed by an Asp at its last two positions. A general formula for characterizing a WD40 repeat is

$\{X_{6-94}[\text{GH-X}_{23-41}\text{-WD}]\}_N$ (SEQ ID NO: 3)

wherein X_{6-94} represents from 6 to 94 contiguous amino acid residues, X_{23-41} represents from 23 to 41 contiguous amino acid residues, and N represents an integer from 4-8 (Neer et al., *Nature* 371:297 (1994)). Other WD40 repeats will, however, be appreciated by those skilled in the art. The number of WD-40 repeats in a particular protein can range from two to more than eight.

Replacement claims with changed marked thereon:

1. (Amended) An isolated and/or recombinant cell- or tissue-specific F-box protein having an amino acid sequence that is at least 75% identical or homologous to SEQ ID NO: 2.
9. (Amended) The isolated and/or recombinant cell- or tissue-specific F-box protein of claim 1, which polypeptide is at least [75] 90 % homologous to the amino acid sequence [shown in Figure 5B] set forth in SEQ ID NO: 2.
13. (Amended) An isolated nucleic acid comprising a nucleotide sequence encoding a cell- or tissue-specific F-box polypeptide of claim 3 or a portion thereof, or a nucleotide sequence complementary thereto [, said F-box polypeptide including an amino acid sequence identical or homologous to the amino acid sequence set forth in Figure 5B, or a portion thereof].
14. (Amended) [The] An isolated nucleic acid [of claim 13, which nucleic acid] that hybridizes under stringent conditions to a nucleic acid [probe] comprising [a] nucleotide sequence [represented by at least 60 consecutive nucleotides of the sequence shown in Figure 5A] set forth in SEQ ID NO: 1, or a sequence complementary thereto.
16. (Amended) An isolated nucleic acid of claim 14 comprising a nucleotide sequence encoding a vertebrate cell- or tissue-specific F-box polypeptide.
17. (Amended) The nucleic acid of claim [13 or 16] 14, further comprising a transcriptional regulatory sequence operably linked to said nucleotide sequence so as to render said nucleic acid suitable for use as an expression vector.

21. (Amended) A transgenic animal having cells which harbor a transgene comprising the nucleic acid of claim [13 or 16] 14, or in which a gene comprising said nucleic acid is disrupted.
22. (Amended) [An] The isolated nucleic acid of claim of claim 14 which selectively hybridizes under high stringency conditions to at least ten nucleotides of the sequence set forth in Figure 5A or complementary sequences thereof, which nucleic acid can specifically detect or amplify a nucleic acid sequence of a vertebrate cell- or tissue-specific F-box gene.
24. (Amended) A reconstituted protein mixture comprising [a] the cell- or tissue-specific F-box polypeptide of claim 1 and a substrate protein.
25. (Amended) The reconstituted protein mixture of claim 24, wherein the cell- or tissue-specific F-box polypeptide is atrophin-1 comprising SEQ ID NO: 2.
27. (Amended) An assay for identifying an inhibitor of cell- or tissue-specific F-box protein-mediated ubiquitination, comprising:
 - (i) providing a ubiquitin-conjugating system including a substrate polypeptide, an SCF complex including one or more cell- or tissue-specific F-box polypeptides of claim 1 and ubiquitin, under conditions which promote ubiquitination of the substrate polypeptide by the SCF complex;
 - (ii) contacting the ubiquitin-conjugating system with a candidate agent;
 - (iii) measuring a level of ubiquitination of the substrate polypeptide in the presence of the candidate agent; and
 - (iv) comparing the measured level of ubiquitination in the presence of the candidate agent with ubiquitination of the substrate polypeptide in the absence of the candidate agent,wherein a statistically significant decrease in ubiquitination of the substrate polypeptide in the presence of the candidate agent is indicative of an inhibitor of cell- or tissue-specific F-box protein-mediated ubiquitination.
42. (Amended) An assay for identifying an inhibitor of an interaction between a substrate polypeptide and an SCF complex including a cell- or tissue-specific F-box protein, comprising:

- (i) providing a reaction system including the substrate polypeptide and an SCF complex including a cell- or tissue-specific F-box protein of claim 1, wherein the substrate polypeptide and the SCF complex interact;
 - (ii) contacting the reaction system with a candidate agent;
 - (iii) measuring formation of complexes containing the substrate polypeptide and the SCF complex in the presence of the candidate agent; and
 - (iv) comparing the measured formation of complexes in the presence of the candidate agent with complexes formed in the absence of the candidate agent,
- wherein a statistically significant decrease in the formation of complexes in the presence of the candidate agent is indicative of an inhibitor of the interaction of the substrate polypeptide and the SCF complex.
43. **(Amended)** The assay of claim 42, wherein the cell- or tissue-specific F-box protein is atrophin-1 comprising SEQ ID NO: 2.
50. **(Amended)** A method for diagnosing a muscle wasting disorder in a patient, comprising:
- (i) ascertaining the level of expression of an F-box polypeptide of claim 1 comprising the sequence set forth in Figure 5B in a sample of muscle cells from the patient; and
 - (ii) diagnosing the presence or absence of a muscle wasting disorder utilizing, at least in part, the ascertained level of expression or activity of the F-box polypeptide;
- wherein an increased level of expression of the F-box polypeptide or F-box polypeptide-dependent ubiquitination activity in the sample, relative to a control sample of non-muscle cells, correlates with the presence of a muscle wasting disorder.
54. **(Amended)** A method for treating a [pateint] subject suffering from a muscle wasting disorder or for maintaining or increasing the muscle mass of a subject, comprising administering to the [pateint] subject an amount of an atrophin-1 inhibitor identified by the method of claim 42 effective to inhibit the expression and/or activity of atrophin-1.
58. **(Amended)** A method for stimulating the proliferation of muscle stem cells, comprising contacting the stem cells with a compound capable of inhibiting the expression and/or activity of atrophin-1 and identified according to the method of claim 42.

60. **(Amended)** A method for inhibiting protein degradation in muscle tissue of a patient without [substantially] substantially affecting protein [degradation] degradation in other tissues, [compising] comprising administering to the patient an amount of an atrophin-1 inhibitor identified by the method of claim 42 effective to inhibit the expression and/or activity of atrophin-1.